PB86-217007

Abiotic Reduction Reactions of Anthropogenic Organic Chemicals in Anaerobic Systems: A Critical Review

Colorado School of Mines, Golden

Prepared for

Environmental Research Lab., Athens, GA

1986

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TECHNICAL REPORT DATA (Please read Instructions on the reverse before complet		
1. REPORT NO. EPA/600/J-86/092	2.	3. PB86-217007
4. TITLE AND SUBTITLE ABIOTIC REDUCTION REACTIONS OF ANTHROPOGENIC ORGANIC CHEMICALS IN ANAEROBIC SYSTEMS: A CRITICAL REVIEW (Journal Version)		5. REPORT DATE 1986 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Donald L. Macalady, Paul G. Tratnyek and Timothy J. Grundl		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Chemistry/Geochemistry Colorado School of Mines Golden, CO 80401		10. PROGRAM ELEMENT NO. CBWD1A 11. CONTRACT/GRANT NO. 811250
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory - Athens, GA Office of Research and Development U.S. Environmental Protection Agency Athens, GA 30613		13. TYPE OF REPORT AND PERIOD COVERED Journal article 14. SPONSORING AGENCY CODE EPA/600/01
15. SUPPLEMENTARY NOTES Published in: Journal of Contaminant Hydrology. 1(1/2):1-28, February 1986		

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

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NATIONAL TECHNICAL
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SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	30
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

EPA/600/J-86/092
JOURNAL ARTICLE

Journal of Contaminant Hydrology, 1 (1986) 1—28
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

Review Paper

ABIOTIC REDUCTION REACTIONS OF ANTHROPOGENIC ORGANIC CHEMICALS IN ANAEROBIC SYSTEMS: A CRITICAL REVIEW

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(Received March 15, 1985; revised and accepted October 1, 1985)

ABSTRACT

Macalady, D.L., Tratnyek, P.G. and Grundl, T.J., 1986. Abiotic reduction reactions of anthropogenic organic chemicals in anaerobic systems: A critical review. In: D.L. Macalady (Editor), Transport and Transformations of Organic Contaminants. J. Contam. Hydrol., 1: 1—28.

This review is predicated upon the need for a detailed *process-level* understanding of factors influencing the reduction of anthropogenic organic chemicals in natural aquatic systems. In particular, abiotic reductions of anthropogenic organic chemicals are reviewed. The most important reductive reaction is alkyl dehalogenation (replacement of chloride with hydrogen) which occurs in organisms, sediments, sewage sludge, and reduced iron porphyrin model systems. An abiotic mechanism involving a free radical intermediate has been proposed. The abstraction of vicinal dihalides (also termed dehalogenation) is another reduction that may have an abiotic component in natural systems. Reductive dehalogenation of aryl halides has recently been reported and further study of this reaction is needed. Several other degradation reactions of organohalides that occur in anaerobic environments are mentioned, the most important of which is dehydrohalogenation. The reduction of nitro groups to amines has also been thoroughly studied. The reactions can occur abiotically, and are affected by the redox conditions of the experimental system. However, a relationship between nitro-reduction rate and measured redox potential has not been clearly established. Reductive dealkylation of the N- and O-heteroatom of hydrocarbon pollutants has been observed but not investigated in detail. Azo compounds can be reduced to their hydrazo derivatives and a thorough study of this reaction indicates that it can be caused by extracellular electron transfer agents. Quinone—hydroquinone couples are important reactive groups in humic materials and similar structures in resazurin and indigo carmine make them useful as models for environmental redox conditions. The interconversion of sulfones, sulfoxides, and sulfides is a redox process and is implicated in the degradation of several pesticides though the reactions need more study. Two reductive heterocyclic cleavage reactions are also mentioned. Finally, several difficulties (both semantic and experimental) that recur in the studies reviewed are discussed. The subtle effects of various sterilization techniques on extracellular biochemicals and complex chemical reducing agents in sediment have stifled attempts to separate abiotic from biological degradation reactions. The characterization of redox conditions in a natural system is still problematic since measured redox potential is not adequate. Suggestions for future research toward a process-level understanding of abiotic chemical reductions are made.

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INTRODUCTION

Anthropogenic chemicals are introduced into surface and groundwaters from a variety of sources including spills, pesticides leached from agricultural land, leaking waste disposal sites, and effluent from municipal and industrial waste treatment facilities. Because these chemicals may threaten ecosystems and their human inhabitants, considerable effort has been expended over the past twenty years to understand the fates of such chemicals (see for example Morrill et al., 1982; Gibson, 1984; Bitton and Gerba, 1984; Ward et al., 1985).

Early efforts to understand transport and transformation phenomena were largely *system-level* investigations. Such studies document the fate of *particular* anthropogenic chemicals in a *particular* environmental system such as a lake, pond, agricultural field, aquifer, or on a model system such as a soil column, small experimental pond, or laboratory microcosm. In addition to providing the information necessary to deal with particular cases of anthropogenic pollution, system-level studies, taken together, form a catalog of natural transport processes, degradation pathways (their rates and products), and influential physico-chemical properties from which a *process-level* understanding can be developed. It is this more general understanding that provides for flexible models that can be used to predict transport and transformations of anthropogenic chemicals in a variety of natural systems. Thus, the emphasis of recent research has largely shifted toward developing an understanding of the actual *processes* (e.g., sorption, transfomations due to microbial activity, surface catalysis, solubilization by dissolved humic substances, and abiotic chemical reactions) which govern the overall system-level phenomena which have been observed or are to be predicted. This type of understanding is essential for the success of any corrective or preventative measures undertaken to alleviate contamination of aquatic systems with anthropogenic organic chemicals.

Considerable progress has been made toward a process-level understanding of *aerobic* aquatic systems. Biological transformations in surface waters have been extensively characterized (Baughman et al., 1980; Paris et al., 1981; Hutzinger, 1982). Of abiotic chemical processes in aerobic systems, hydrolysis reactions are perhaps the best understood (Mabey and Mill, 1978; Wolfe, 1980; Macalady and Wolfe, 1983, 1984, 1985; Perdue and Wolfe, 1983). Oxidation (Goring et al., 1975; Mill et al., 1982) and photolysis (Zepp and Cline, 1977; Mill et al., 1980; Zepp, 1982) reactions have also been investigated. Physical processes that have been studied in detail include volatilization, sorption, and surface catalysis (Hoffman, 1980; Roberts et al., 1980; McCarty et al., 1981; Mill et al., 1982; Karickhoff, 1984; Coates and Elzerman, this volume). Computer models have been developed to apply most of this process-level knowledge in simulations of transport and transformation of organics in surface waters (Burns, 1983).

The development of a process-level understanding of transport and transformation phenomena for organic chemicals in *anaerobic* aquatic systems is

at a much less advanced stage. This lack of understanding is disturbing because anaerobic environments comprise a major portion of the natural hydrosphere including, for fresh waters, most sediments and muds, many groundwater systems, and the seasonally anaerobic waters of many lakes and reservoirs. Anaerobic conditions also pertain to certain types of agriculture (e.g. rice) and portions of many waste treatment regimes.

It is the purpose of this review to focus on one particular process involving organic chemicals in anaerobic aquatic systems, *abiotic reduction reactions*, and to critically review our present understanding of such processes.

It is important to recognize the ambiguity that may be engendered by the term "abiotic". There is a spectrum of phenomena between those that are strictly biological and those that involve only non-biological reactants. This can lead to semantic confusion. For the purpose of this review, the most restricted and literal definition of abiotic will be adopted: All processes not directly involving the participation of metabolically active microorganisms. This issue is an important one since the term abiotic, as defined here, encompasses a host of processes mediated by compounds generally associated with biological activity, but not necessarily directly involving active microorganisms. Such compounds may include extracellular enzymes, NADH, NADPH, flavins, flavoproteins, hemoprotein, iron porphyrins, chlorophylls, cytochromes, and glutathiones (Bouwer and McCarty, 1983a). Surfaces of disrupted biological membranes may also be involved.

Another potential semantic confusion relates to the meaning of the term anaerobic. Literally, this term refers to environments devoid of measurable free oxygen and in particular to those environments unable to support microorganisms which use oxygen as an electron acceptor (Singleton and Sainsbury, 1978). However, the term fails to differentiate a considerable range of chemical conditions which exist in such environments. It is the conditions present, such as speciation, activity, and catalytic characteristics of organic and metallic reducing agents, not the absence of free oxygen or aerobic microorganisms per se which control the abiotic reduction reactions of organic chemicals in a particular natural environment. The problems (conceptual and analytical) associated with the unambiguous and chemically accurate characterization of this range of conditions are formidable.

System-level investigations of reductive transformations generally do not effectively distinguish between biological and abiotic processes. In laboratory studies, autoclaved systems have frequently been used to establish the biological origin of observed reactivity. However, the absence of reductive activity in autoclaved controls does not necessarily establish that such reactions require living microorganisms. Autoclaving alters reaction systems in poorly characterized ways, including possible destruction of the catalytic activity of extracellular biomolecules or solid surfaces which may mediate the reactions observed in untreated systems. Also, the restoration of reactivity when autoclaved systems are inoculated with microorganisms does not establish the direct role of microbes. Inoculation may restore

activity by microbial reduction of soil materials which in turn are active in degradation reactions (Glass, 1972). Chemical sterilants and inactivating substances have also been used to prevent microbial activity in reaction systems. Formaldehyde, mercuric chloride, m-cresol, and sodium azide are common agents for this purpose (Brock, 1978). Though the modes of action of such chemical poisons may be understood, their effects on the complex chemical systems present in natural aquatic environments are poorly defined. In light of these difficulties, a comprehensive consideration of the reductive reactions which may be abiotic must include many reactions currently considered to be caused by biological activity.

Several reviews of organic reactions in anaerobic systems have been published (e.g., Sethunathan, 1973; Williams, 1977; Tsukano, this volume) and anaerobic reactions have been included in more general reviews of transformation reactions (e.g. Morrill et al., 1982). Williams (1977) summarized observed reactions according to the organic functional groups involved. This systematic approach is appropriate for a general review of reduction reactions in anaerobic environments and will be adopted here.

The following discussion includes detailed reviews of the literature on dehalogenation and nitro-reduction. Less detailed discussions of other reduction reactions which have been observed in anaerobic systems are also presented, including dealkylation, azo-reduction, quinone—hydroquinone interconversion, reduction of sulfoxides and sulfones, and heterocyclic ring cleavage. This is followed by a general summary of factors influencing abiotic reduction processes in natural aquatic systems and our recommendations for future investigations.

DEHALOGENATION REACTIONS

Chlorinated hydrocarbons, are by far the most widespread class of anthropogenic organohalide pollutants and will be emphasized in this review. Included are DDT and its analogs, hexachlorocyclohexanes (especially gamma-BHC, lindane), polychlorobornanes (e.g. the mixture of chlorinated camphenes known as toxaphene), dimers of hexachlorocyclopentadiene e.g. mirex), chlorinated aromatics [e.g. 2,4-dichlorophenoxyacetic acid (2,4-D)], simple chlorinated alkanes (e.g. 1,1,2-trichloroethane), and the cyclodienes: chlordane (1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene), heptachlor (1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene), aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo,exo-5,8-dimethanonaphthalene), dieldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-exo-6,7-epoxy-1,4-endo,exo-5,8-dimethanonaphthalene) and endrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-epoxy-1,4-endo,endo-5,8-dimethanonaphthalene). The reactive functional groups of concern are alkyl and aryl chlorides.

One of the earliest and most comprehensive studies on anaerobic degradation of chlorohydrocarbons is that of Hill and McCarty (1967). These

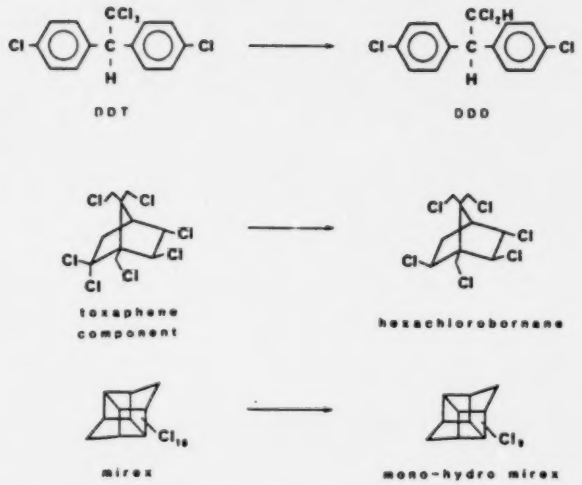

Fig. 1. Molecular structures of reactants and products for the reductive dechlorination of DDT, toxaphene components, and mirex. See text for references.

authors presented considerable evidence to dispel the notion that organic compounds which are highly persistent in aerobic environments will also be recalcitrant to anaerobic degradation. They observed that gamma-BHC, heptachlor, endrin, DDT, DDD and aldrin all degrade in anaerobic sewage sludge. The data indicated that the observed degradation was primarily due to microbial activity and no rigorous attempt was made to determine how much reactivity was due to abiotic processes. The possibility that extracellular enzymes may contribute to the degradation was acknowledged. Since then, Guenzi et al. (1971) published a wide ranging study that showed that flooded soils (in particular those with measured redox potentials at or below + 250 mV) favor the degradation of heptachlor, DDT, gamma-BHC, and endrin.

The most thoroughly studied degradation reaction of the halogenated hydrocarbons is reductive dehalogenation: The replacement of an alkyl halide with hydrogen. An example of this is the conversion of DDT to DDD (Fig. 1). Reductive dehalogenation of DDT has been observed to be the primary degradation pathway in anaerobic soil, sediment, and sewage waste (Hill and McCarty, 1967; Guenzi and Beard, 1967; Burge, 1971; Albone et al., 1972; Jensen et al., 1972; Zoro et al., 1974). Toxaphene components degrade in a variety of model environmental systems at the geminal-dichloro groups by reductive dechlorination (Khalifa et al., 1976; Saleh et al., 1977; Fig. 1). Mirex undergoes reductive dechlorination by iron(II) porphyrin to the mono-, di-, tri-, and tetra-hydro derivatives (Holmstead, 1976; Fig. 1). Reductive dechlorination has also been observed in halogenated methanes and ethanes such as carbon tetrachloride (Bouwer and McCarty, 1983a, b;

Klecka and Gonsior, 1984), chloroform (Klecka and Gonsior, 1984), 1,1,2-trichloroethane (Kleopfer et al., 1985), and 1,2 dibromoethane (Bouwer and McCarty, 1985). Recent studies have appeared that indicate that even polychlorinated biphenyls are reductively dechlorinated in anaerobic sediments (Brown et al., 1985).

Experiments that help to differentiate abiotic from microbial processes in reductive dechlorination fall into two categories: those involving sterilized sediment, soil, or sewage sludge and those involving model environmental systems. The former are typically inconclusive: For example, the inhibition of reductive dechlorination in soils by autoclaving has been observed by Guenzi and Beard (1967), Ko and Lockwood (1968), and Burge (1971). However, as Burge noted, that is equivocal evidence that the reaction is performed by microbes because autoclaving may inactivate other reducing agents such as free radicals in humic materials, ferrous iron, porphyrins, or extracellular enzymes. Burge went on to conclude that the reaction is "definitely microbial" because inoculation of autoclaved soil with a small amount of untreated soil restores the capacity to reduce DDT. However, other studies indicate that the restoration of reactivity in autoclaved soil by inoculation may not be due to direct microbial mediation, but rather to reduction of abiotic soil materials by microorganisms which in turn reduce the substrate (Glass, 1972).

The second type of experiment that has produced evidence for abiotic reductive dechlorination makes use of model environmental systems. Several of these have been used to model the types of reducing agents presumed to be active in natural soils and sediments. The reductive dechlorination of DDT to DDD has been observed in solutions of iron(II) porphyrin (Castro, 1964; Miskus et al., 1965; Wade and Castro, 1973a, b; Zoro et al., 1974; Marei et al., 1978; Quirke et al., 1979). Abiotic reductive dechlorination of mirex by iron(II) porphyrin has been reported (Holmstead, 1976). Glass (1972) used a $Fe_3(OH)_8$ precipitate on autoclaved quartz sand as a model system for reducing sediment. He observed conversion of DDT to DDD and attributed it to reaction with the species $Fe_3(OH)_8$-Fe^{2+}. Reductive dechlorination of some chlorinated methanes and ethanes was observed in redox poising solutions of Na_2S, cysteine, Na_2S/Fe(II) porphyrin, and cysteine/Fe(II) porphyrin (Klecka and Gonsior, 1984). Electrochemical reductive dechlorination has been observed for DDT (Rosenthal and Lacoste, 1959), chlorinated benzenes (Farwell et al., 1975a), and polychlorinated biphenyls (Farwell et al., 1975b).

In general, reductive dechlorination can occur by either nucleophilic or free radical substitution (March, 1985) but all the evidence from natural or model anaerobic systems supports a free radical mechanism. The details of this mechanism for abiotic reductive dechlorination of alkyl chlorides are found in Wade and Castro (1973a, b). The mechanism they proposed is analogous to that by which organic halides oxidize Cr(II). The first step (Fig. 2) is the formation of a complex between Fe(II) of the porphyrin and the

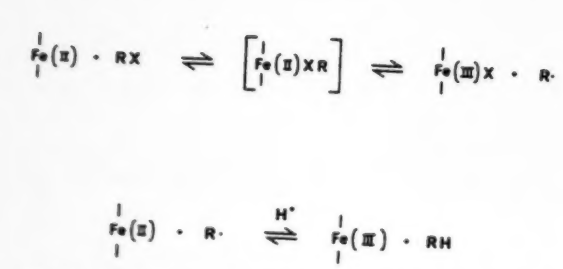

Fig. 2. Proposed pathway for the reduction of alkyl halides (RX) by iron(II) porphyrin (Wade and Castro, 1973 a, b). Only the Fe(II) portion of the chelate structure is shown.

alkyl halide. This complex facilitates a one-electron transfer leaving an alkyl radical and an oxidized iron porphyrin chloride. This second step is rate controlling. In the third and fourth steps, the alkyl free radical is quenched with an electron from another Fe(II) porphyrin and a proton from solution. Alternatively, Ahr et al. (1980, 1982) have proposed that the free radical intermediate abstracts a hydrogen atom directly from its surroundings. Glass (1972) has described how the free radical reduction of DDT to DDD by Fe(II) porphyrins may be coupled with the oxidation of reduced organic matter produced by microbial respiration (Fig. 3). This, in effect, makes the iron porphyrin a mediator of the electron transfer from reduced organic matter to the alkyl halide. Saleh et al. (1977) have determined that the 2-endo and 2-exo monochloro derivatives of toxaphene components are formed by reductive dechlorination with iron porphyrin. This lack of stereospecificity may indicate the free radical mechanism (Saleh et al., 1977). Klecka and Gonsior (1984) have hypothesized that other electron carriers of biological origin such as cytochromes, flavoproteins, chlorophylls, iron-sulphur proteins, and enzymes may perform in a manner similar to iron(II) porphyrin in degrading chlorohydrocarbons.

The only rigorous studies of the kinetics of reductive dechlorination are reported by Wade and Castro (1973 a, b). The rate of appearance of the iron(III) porphyrin was monitored spectrophotometrically. The observed rate law for the reductive dechlorination of alkyl halides was: rate = k[Fe(II) porphyrin]^2[RX], where $k = 8 \times 10^3$ mole^2 s^{-1}. Hill and McCarty (1967) speculated from their data that reductive dechlorination is first-order in DDT. Glass (1972) noted that the rate of conversion of DDT to DDD varies with the redox potential of the system. The data of Klecka and Gonsior (1984) suggest the rate of reductive dechlorination of carbon tetrachloride in

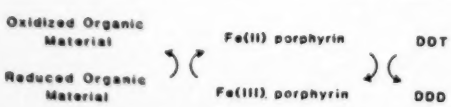

Fig. 3. Coupled redox cycles providing for abiotic reductive dehalogenation of chlorinated hydrocarbons such as DDT (Glass, 1972).

a Na_2S redox poising solution is proportional to the log of iron porphyrin concentration. However, the reaction rate was independent of sulfide ion concentration.

The term reductive dechlorination has also been used to refer to the removal of vicinal chlorides, resulting in the formation of a carbon—carbon double bond on the substrate (Tsukano and Kobayashi, 1972; Ohisa et al., 1980; Tsukano, this volume). This reaction has been observed for gamma-BHC in flooded rice soils (Tsukano and Kobayashi, 1972), anaerobic sewage sludge (Beland et al., 1976), cell-free extracts of *Clostridium rectum* (Ohisa et al., 1980), and at a platinum cathode (Beland et al., 1976; Kurihara et al., 1981; Fig. 4). Kurihara et al. (1981) have partially isolated the reducing agent responsible for vicinial dechlorination in cell-free extracts of *Clostridium rectum*. Beland et al. (1974) observed that electrochemical reduction caused this reaction for DTE (an analog of DDT; Fig. 4). Reduction of hexachloroethane to tetrachloroethylene has been demonstrated in iron(II) porphyrin model systems (Wade and Castro, 1973b) and groundwater (Criddle et al., this volume). It is apparent that vicinal chloride elimination can occur without direct involvement of living organisms. The mechanism has not been studied in environmental systems and may be complex since it depends on the reactants and reaction conditions (March, 1985).

Until recently, only alkyl chlorides were known to undergo reduction in natural anaerobic systems. However, Sulflita et al. (1982) have shown that halogenated benzoates such as chloramben, 2,3,6-trichlorobenzoic acid, and dicamba (Fig. 5) undergo dechlorination in anaerobic sewage and lake sediment. They concluded that this reaction requires direct microbial mediation. Since then, Sulflita et al. (1984) have reported that a culture of anaerobic methanogens can cause dechlorination of 2,4,5-T to 2,4-D (Fig. 5). Dechlorinations of 2,4,5-T, 2,4-D, pentachlorophenol, 2,4,6-trichlorophenol, and 2-, 3-, and 4-chlorophenol have been reported in anaerobic sewage sludge (Mikesell and Boyd, 1985). They observed that removal of

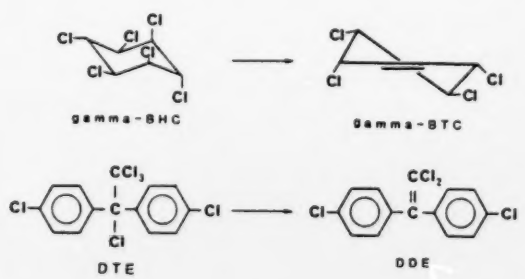

Fig. 4. Molecular structures of the reactants and products for the vicinal chloride elimination reactions of gamma-BHC and the DDT derivative, DTE. See text for references.

chlorine atoms ortho to the phenolic group is favored. Kuwatsuka (1977) detected dechlorination at the meta and para positions of pentachlorophenol in sterilized flooded soil. This abiotic aryl dechlorination is somewhat remarkable in view of the fact that such reactions occur only under relatively extreme reducing conditions in laboratory systems (March, 1985).

Several related degradation reactions which are closely associated with anaerobic environments will be mentioned briefly. Dehydrochlorination is an important degradation reaction of chlorohydrocarbons that involves removal of adjacent hydrogen and chlorine atoms leaving a carbon—carbon double bond on the substrate. Dehydrochlorination of DDT to DDE (Fig. 6) has been observed in natural anaerobic environments and reduced porphyrin model systems (Guenzi and Beard, 1967; Burge, 1971; Marei et al., 1978; Quirke et al., 1979). 1,1,2,2-tetrachloroethane undergoes dehydrochlorination and the reaction is unaffected by redox poisers or iron porphyrin (Klecka and Gonsior, 1984). The dehydrohalogenation of gamma-BHC to gamma-pentachlorocyclohexane has been observed in flooded soils (Yule et al., 1967). The presence of DDMU (Fig. 7) among the anaerobic degradation products of DDT has been attributed to dehydrohalogenation of DDD (Quirke et al., 1979). The mechanism of dehydrohalogenation of DDT to DDE may be base-catalyzed or result from the loss of H · by the same free radical intermediate proposed for the reductive dechlorination to DDD (Quirke et al., 1979; Fig. 6).

In addition to DDD and DDE, there are several other chlorinated

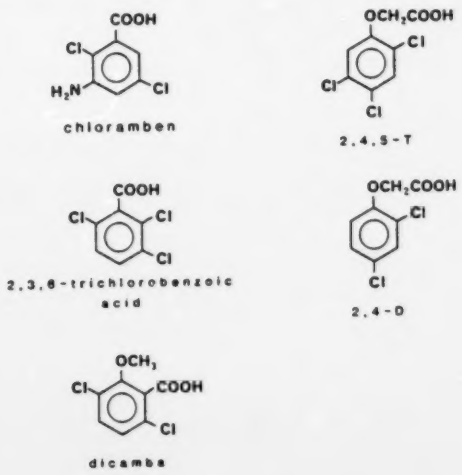

Fig. 5. Molecular structures for chloramben, 2,3,6-trichlorobenzoic acid, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,4-dichlorophenoxyacetic acid (2,4-D), and dicamba.

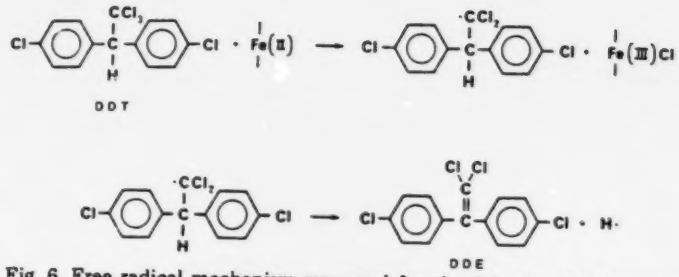

Fig. 6. Free radical mechanism proposed for the dehydrochlorination of DDT by iron(II) porphyrins by Quirke et al. (1979). Only the Fe(II) portion of the chelate is shown.

hydrocarbons that result from the anaerobic degradation of DDT. These substances are rarely detected because of their low concentration, their polarity (which necessitates esterification prior to analysis by gas chromatography), and their relatively low response in an electron capture detector (Marei et al., 1978). Guenzi and Beard (1967) detected DDA, BA, dicofol, DBP, and DBM (Fig. 7) as trace decomposition products of DDT in

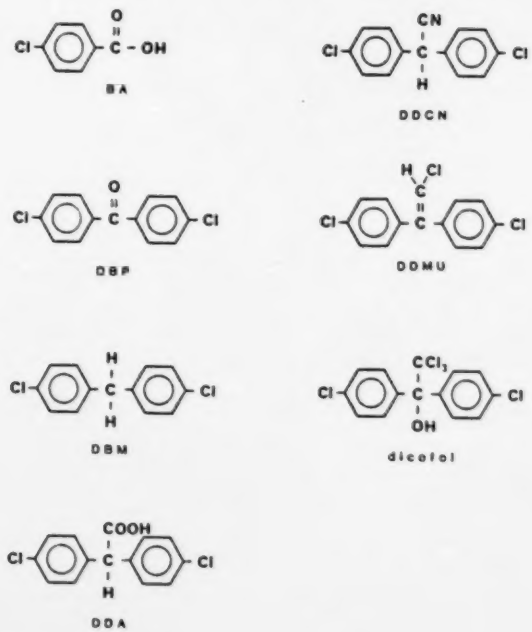

Fig. 7. Molecular structures for minor products observed in the anaerobic degradation of DDT. See text for references.

anaerobic soil cultures. These were separated by two-dimensional thin-layer chromatography and identified by comparison with standard authentic samples. Jensen et al. (1972) detected traces of DDMU, DDCN, and DBP (Fig. 7) in sewage sludge after incubation with DDT. Marei et al. (1978) and Quirke et al. (1979) observed traces of DDCN, DBP, DDMU, and DDA in solutions of DDT, reduced iron porphyrin and ammonia (Fig. 7).

NITRO REDUCTION

The reduction of nitro groups to amines is a widely observed degradation pathway for anthropogenic chemicals in anaerobic environments. It has been studied for three groups of anthropogenic organic chemicals: the dinitroaniline herbicides {e.g., trifluralin, and benefin [N-butyl-N-ethyl-2,6-dinitro-4-(trifluoromethyl)benzenamine]}, nitro-containing organophosphorous pesticides [e.g., parathion, and methylparathion (O,O-dimethyl O-4-nitrophenyl phosphorothioate)], and chlorinated nitrobenzenes (Figs. 8 and 9). Nitro reduction is generally postulated to be the first in a series of degradation reactions leading to complete mineralization of many nitro-containing anthropogenic organic compounds. Probst et al. (1967) proposed a pathway for anaerobic trifluralin degradation that involves nitro reduction of one group followed either by dealkylation or reduction of the second nitro group (Fig. 9). In natural systems, reduction of the second nitro group has not been observed (Willis et al., 1974; Helling, 1976; Southwick and Willis, 1979). It should be noted that complete mineralization to CO_2 by microorganisms often follows reduction, dealkylation, or hydrolysis reactions. However, the ring cleavage required for mineralization of the nitro compounds mentioned above is reported to be strongly inhibited under reducing conditions (Goswami and Green, 1971; Helling, 1976; Gambrell et al., 1983).

Several authors have argued that nitro reduction occurs abiotically. The evidence presented in support of abiotic nitro reduction includes degradation rates which are presumed too fast to be microbial (Wahid et al., 1980; Gambrell et al., 1983), degradation in sterile systems (Southwick and Willis, 1979; Wahid et al., 1980), and the lack of correlation between degradation rate and CO_2 production (Gambrell et al., 1983). Southwick et al. (1976) postulated an abiotic pathway for the initial steps in the nitro reduction of dinitroaniline herbicides based on their relative ease of polarographic reduction.

It is probable that metabolic activity by anaerobic microorganisms is necessary to create the conditions for abiotic nitro reduction. Nitro reduction requires that the standard potential of the reducing agent be more negative than that of the substrate nitro group. Few investigators of nitro reduction characterize the redox state of their system in more detail than "anaerobic". However, some have reported the relationship between

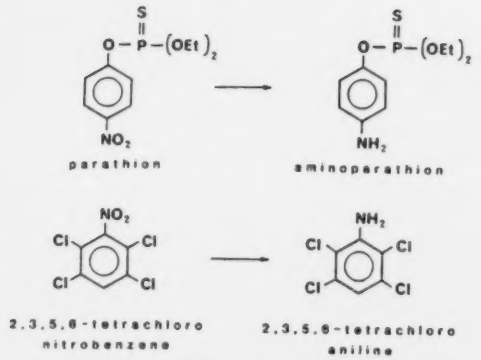

Fig. 8. Reactants and products for nitro reduction reactions of parathion and 2,3,5,6-tetrachloronitrobenzene.

measured redox potential (Eh) and reaction rate. Willis et al. (1974) observed a threshold value of approximately +100 mV (versus standard hydrogen electrode, SHE) above which degradation of trifluralin was sharply inhibited. Geer (1978) observed a threshold of −160 mV (SHE) for nitro reduction of 2,3,5,6-tetrachloronitrobenzene. Gambrell et al. (1983) observed that the rate of methylparathion reduction increased as redox potential became more negative, although no clear-cut threshold potential was indicated. Regardless of whether or not a threshold potential exists,

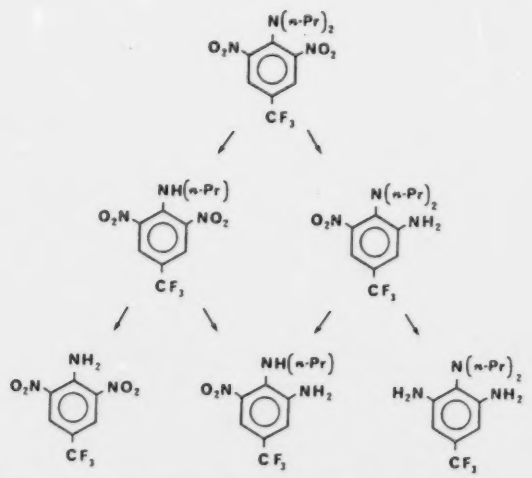

Fig. 9. Degradation pathways for the herbicide trifluralin in anaerobic systems. Proposed by Probst et al. (1967). The pathways on the left and right refer respectively to observations in aerobic and anaerobic aquatic systems.

measured redox potential of the reaction medium is an unreliable indicator of its ability to cause nitro reduction (Geer, 1978). The fact that nitro reduction occurs in systems with measured redox potentials substantially above the standard redox potential of the nitro group sustantiates this unreliability. [The standard potential for nitro reduction is highly pH dependent and compound specific. Values for the dinitroaniline herbicides are between −100 and −300 mV (SHE) at a pH of 5.1. Trifluralin has redox potentials of −190 and −300 mV (SHE) at pH 5.1 and 7.4, respectively (calculated from data in Southwick et al., 1976).] Measurements of Eh are apparently controlled by redox couples that are not important determinants of the rate of nitro reduction. Measured redox potential is therefore only a qualitative indicator of a system's ability to effect nitro reduction.

Process-level understanding of nitro reduction requires identification and quantification of reducing agent(s) responsible for the degradation reaction(s). Wade et al. (1969) and Zoro et al. (1974) showed that free Fe(II) porphyrins are capable of performing nitro reduction. Geer (1978) adopted this idea to explain the nitro reduction observed in anaerobic sewage sludge. Extracellular enzymatic mediation of nitro reduction is suggested in the work reported by Wahid et al. (1980) in which parathion degradation was studied in autoclaved and gamma-irradiated soils. Autoclaved soils, in which all enzymatic and biological activity was presumed destroyed, exhibited virtually no degradation, whereas irradiated soils, in which they presumed enzymatic activity continues, exhibited continued parathion degradation.

Southwick and Willis (1979) reported results of a preliminary investigation of nitro reduction considering ferrous iron, sulfide, and phenolic compounds as possible reducing agents. Based on the similarities in degradation rate and product composition of trifluralin in a model $FeSO_4$—NaOH—sand system and a sterilized flooded soil, ferrous iron may be a sufficient reducing agent. Tsukano (this volume) has reported reduction of chlornitrofen (4-nitrophenyl 2,4,6-trichlorophenyl ether) in which ferrous compounds were also implicated as the reducing agents. It is obvious that pH and complexation with organic substances affect the ability of ferrous iron to act as a reducing agent and these reactions will need to be investigated further.

Phenolic compounds may also serve as reducing agents. Southwick and Willis (1979) observed trifluralin degradation by gallic acid (3,4,5-trihydroxybenzoic acid). In this case the usual amino derivatives (Fig. 9) were not found and the hydroxylamine had been proposed as the product. In the same study, sulfide was found to be insignificant in the reduction of trifluralin. The authors attributed this observation to trifluralin/sulfide steric interferences and the precipitation of sulfide as FeS. Whether or not sulfide acts as a reducing agent for other sterically unhindered organic nitro compounds or in an iron-poor environment is not known.

Geer (1978) tested the hypothesis that the standard redox potential of the

nitro group to be reduced can be used to predict its fate in anaerobic waste digestor sludge. He concluded, however, that this was not a useful approach and attention to classification of reducible functional groups is more appropriate.

Published data on the kinetics of nitro reduction is generally system-specific and not quantitative: Rate constants or half-lives are rarely reported. Pseudo first-order kinetics have been assumed in all cases. Complete degradation of dinitroaniline herbicides has been observed to occur within several days in soils with a negative potential (Willis et al., 1974; Southwick and Willis, 1979). The only rate constant reported for a dinitroaniline is $k_{obs} = 0.26\,h^{-1}$ for bifenox [methyl 5-(2,4-dichlorophenoxy)-2-chloronitrobenzoate] in sewage sludge at $-240\,mV$ (SHE; Geer, 1978). Geer also reported a rate constant for 2,3,5,6-tetrachloronitrobenzene of $0.53\,h^{-1}$ in the same system. Qualitative estimates of the half-lives of several organophosphates containing nitro groups have been made and compared to measured redox potentials of the medium. Parathion in soil at $-200\,mV$ (SHE) has a half-life of less than a minute (Wahid et al., 1980). Methyl parathion in soil has a half-life of hours at $-150\,mV$ (SHE) to days at $+450$ mV (SHE, Gambrell et al., 1983). Similar ranges in first-order reduction rates have been observed in our laboratories (unpublished results), but no clear

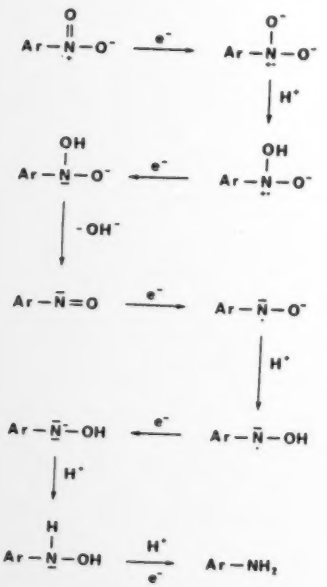

Fig. 10. A proposed mechanism for the reduction of aryl nitro groups to amines (March, 1985).

dependence on the measured redox potential of the sediment—water systems has been demonstrated.

The detailed mechanism of nitro reduction has not been established but is usually presumed to involve single electron additions with nitroso compounds and hydroxylamines as intermediates (March, 1985; Fig. 10). Differences are to be expected depending on the reducing agent. No study of the mechanism of nitro reduction of organic pollutants by natural reducing systems has been reported.

DEALKYLATION

N-dealkylation is the replacement of an alkyl substituent on the nitrogen of an amine or amide by hydrogen. This is a common metabolic pathway and has been observed for carbofuran (Fig. 11; Hassall, 1982), diuron (Fig. 11; Ernst and Boehme, 1965), and trifluralin (Probst and Tepe, 1969). Further studies of anaerobic dealkylation on trifluralin (Williams and Feil, 1971; Parr and Smith, 1973; Willis et al., 1974) and benefin (Fig. 11; Golab et al., 1969) provide evidence that the reaction is microbial. Williams et al. (1968), and Goswami and Green (1971) have demonstrated N-dealkylation reactions in anaerobic systems (both bovine rumen and soil) for a variety of triazine compounds including atrazine (Fig. 11), and the structurally similar compounds propazine and simazine. These studies show a wide variation in degradation rates. However, such variations are equivocal because degradation in some of the studies was measured by monitoring ^{14}C activity

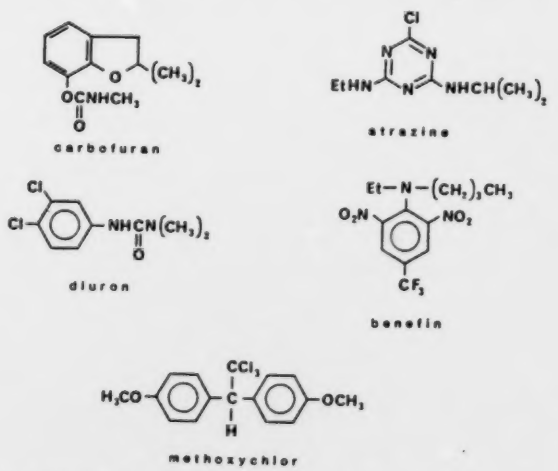

Fig. 11. Molecular structures of the pesticides carbofuran, diuron, benefin, atrazine, and methoxychlor.

of ring-labeled herbicides. This technique measures the entire suite of degeneration reactions not just the dealkylation.

O-dealkylation is the replacement of an alkyl substituent of an ether linkage by hydrogen and has been observed for methoxychlor (Fig. 11; Hassall, 1982). This reaction has not been shown to occur in soil systems. Cleavage of alkoxy groups from compounds such as parathion or methyl-parathion (see Adhya et al., 1981) are hydrolysis reactions and should not be confused with O-dealkylation.

Dealkylations in environmental systems are generally considered secondary degeneration reactions (e.g. Fig. 9) and have not been thoroughly studied. Apparently, neither the agents that effect reductive dealkylation nor the reaction kinetics have been determined. It is not clear whether these reactions can be abiotic.

AZO REDUCTION

Azo reduction is the modification of an azo compound ($R{-}N{=}N{-}R'$) to a hydrazo compound [$R{-}(NH)_2{-}R'$] by the addition of two electrons and two hydrogen ions. Azo compounds are dispersed into the environment through a variety of human activities. The majority of modern food dyes are azo derivatives. Aniline and its derivatives, a major class of industrial chemicals, may condense to form azo compounds under natural conditions (Kaufman, 1970; Pillai et al., 1982). A unique study of the reduction of azo dyes to hydrazo compounds in anaerobic cultures of *Proteus vulgaris* has been performed by Dubin and Wright (1975). This reaction is of interest because the proposed mechanism involves an extracellular "electron shuttle" that mediates the electron transfer between the reducing enzyme(s) of the microorganism and the dye. Dubin and Wright (1975) observed a relationship between the rate of azo dye reduction and redox potential of the dye which indicated that the rate-determining step is a chemical process not a structure specific process (such as transport across a membrane). They also observed that the kinetics of dye reductions were zero-order with respect to dye concentration and concluded that this was an extracellular process. The model they proposed (Fig. 12) for the overall process involves a redox enzyme, E/EH_2, at the cell and a low molecular weight electron carrier (perhaps, for example, flavin), B/BH_2. This work illustrates the mechanistic detail necessary to fully characterize the biological spectrum in natural degradation reactions of anthropogenic pollutants.

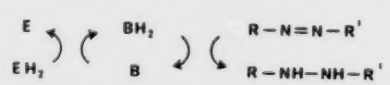

Fig. 12. Redox cycle proposed by Dubin and Wright (1975) for the extracellular reduction of azo dyes by cultures of *Proteus vulgaris*.

QUINONE–HYDROQUINONE INTERCONVERSION

Quinones, semiquinones and hydroquinones are interconverted by the redox reaction shown in Fig. 13. These redox-active functional groups are known to be important in the reactivity of humic substances (Wilson and Weber, 1977; Schnitzer, 1978; Thurman, 1985). However, humic substances are a variable composite of chemical structures and study of the role of individual humic functional groups in the degradation of anthropogenic organic pollutants is extremely difficult. Instead, model compounds with functional groups similar to the quinone–hydroquinone redox couple have been employed. Indigo carmine is a substance which is reduced via a quinone–hydroquinone type of reaction (Fig. 14). Indigo carmine is not a significant pollutant and only one report of its metabolism has been published in which indigo carmine was observed to be reduced by rat gastrointestinal microorganisms (Lethco and Webb, 1966). However, work in our laboratory indicates that indigo carmine and its related sulfonic acid cogeners are promising as prototypes for redox-active substances in natural systems (Tratnyek and Macalady, 1985).

The reduction of resazurin to dihydroresorufin involves a quinone–hydroquinone type redox couple where one keto–enol intercoversion is, instead, an amine-imine interconversion (Fig. 15). The reduction of resazurin

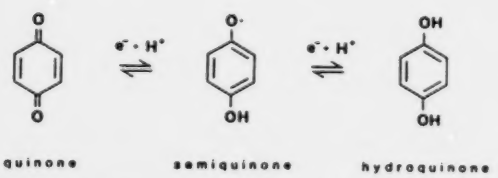

Fig. 13. Redox reactions for the interconversion of quinone, semiquinone, and hydroquinone.

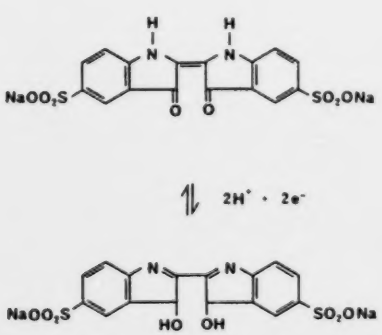

Fig. 14. Redox reaction for indigo carmine and dihydroindigo carmine.

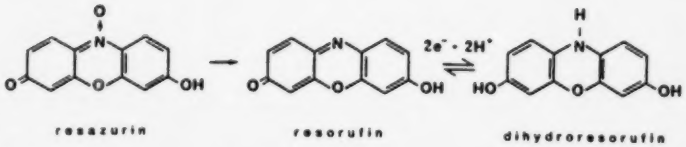

Fig. 15. Redox reactions relating resazurin to resorufin and dihydroresorufin.

in pure cultures of *Flavobacterium sp.* and in sediments is strongly inhibited by the metabolic inhibitor m-cresol (Liu and Strachan, 1979, 1981). This observation has been used as justification for using resazurin reduction as an indicator of microbial metabolism in sediments (Liu and Strachan, 1979, 1981). However, the effect of m-cresol on the various reducing agents in sediment is not known, making the interpretation of such a test difficult.

Recently, aerobic degradation of the insecticide aminocarb [4-(dimethylamino)-3-methylphenyl-N-methylcarbamate] to 1,4-benzoquinones has been observed (Leger and Mallet, 1985). Further alteration by reduction to hydroquinones in anaerobic environments may be a consequential reaction pathway of this (and other) carbamate insecticides (also see Lamberton and Claeys, 1970). The possibility of abiotic reactions in the reduction of anthropogenic quinones in anaerobic aqueous systems has not been systematically evaluated.

SULFOXIDE AND SULFONE REDUCTION

Sulfones, sulfoxides, and sulfides can be interconverted by oxidation—reduction reactions, but evidence for these reactions in natural systems is limited. Walter-Echols and Lichtenstein (1977) reported that phorate sulfoxide (Fig. 16) was reduced to its phorate precursor in lake sediment microcosms. The reaction occurred only very slowly in flooded loam soils, but became more rapid (44% in two weeks) when lake sediment was added. Microbial involvement was indicated by the lack of reduction in autoclaved controls and the increase in reduction rate when the unsterilized microcosms were amended with glucose. Again, this result is equivocal because of the many changes produced in the system by autoclaving. The possible conversion of phorate sulfone to the sulfoxide was also investigated, but no reduction was observed. Gutenmann and Lisk (1970) proposed reductions of the sulfone herbicide nitralin by ruminal fluids (Fig. 17). The insecticide aldicarb (Fig. 17) is rapidly oxidized to the corresponding sulfoxide and sulfone in aerobic soils (Ou et al., 1985a). However, reduction of the sulfoxide and sulfone to aldicarb in anaerobic soils or sediments has not been demonstrated (Smelt et al., 1983; Ou et al., 1985b). Slow (maximum of 12% in 33 days) reduction of the sulfoxide, but not the sulfone, to aldicarb was reported for anaerobic groundwaters amended with glucose and limestone

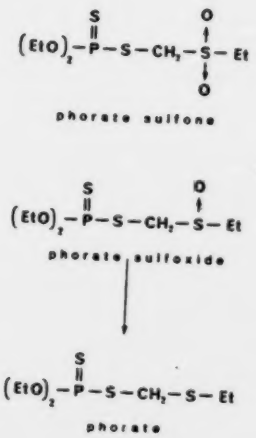

Fig. 16. The structure of phorate and its sulfoxide and sulfone derivatives. Conversion of the sulfoxide to phorate has been observed in anaerobic systems (Walter-Echols and Lichtenstein, 1977).

(Miles and Delfino, 1985). Wolfe (1985) measured the rates of anaerobic degradation of aldicarb, aldicarb sulfoxide and aldicarb sulfone in sterilized and untreated sediment—water systems. Sterilization had little effect on the disappearance rate of the three compounds: half-lives of a few hours were observed. However, aldicarb was apparently not the product of anaerobic degradation of the sulfoxide and sulfone. Because the rates observed were much faster than expected for hydrolysis, some other reaction is implicated.

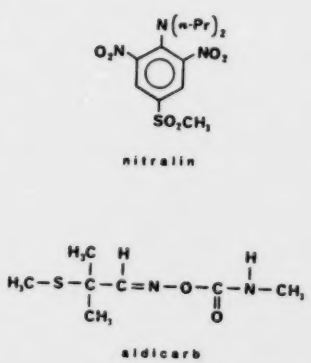

Fig. 17. Molecular structures of the pesticides nitralin and aldicarb.

HETEROCYCLIC RING CLEAVAGE

Some heterocyclic ring cleavage reactions observed in anaerobic systems are also reductions. The reductive decarboxylation of the herbicide methazole (Fig. 18) to CO_2 and 1-(3,4,dichlorophenyl)-3-methylurea is an example (Gutenmann et al., 1972). The degradation of coumarin represents a ring cleavage reaction which has been reported to proceed via a reduced intermediate (Scheline, 1968, Indahl and Scheline, 1971): Coumarin is reduced to the intermediate dihydrocoumarin followed by hydrolytic ring cleavage (Fig. 18). This reductive ring cleavage may have environmental importance since coumarin is an intermediate in the degradation of the widely used insecticide carbaryl (1-napthyl methylcarbamate; Kanzano et al., 1972). However, heterocyclic ring cleavage is apparently an unusual degradation pathway because it has not been observed for most candidates (e.g. triazines, chlorpyrifos, diazinon, paraquat, azinphos methyl, bromacil). No effort has been reported which attempts to determine if reductive heterocyclic ring cleavage has an abiotic component.

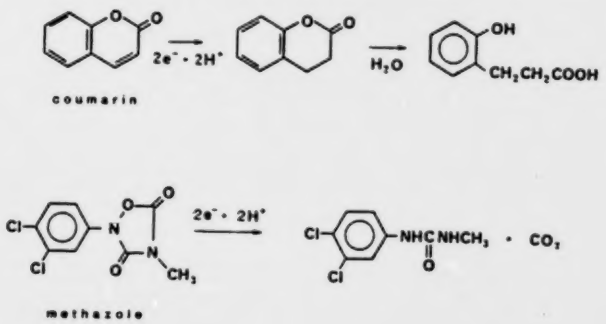

Fig. 18. Reactants and products proposed for heterocyclic ring cleavage reactions of methazole (Gutenmann et al., 1972), and coumarin (Indahl and Scheline, 1971) in anaerobic systems.

SUMMARY

Abiotic reductions of anthropogenic pollutants in natural and model anaerobic systems have been reviewed according to the types of reactions and functional groups involved. In light of the large variety of functional groups liable to undergo reduction reactions in the laboratory (March, 1985), it is likely that additional reaction types occur in natural systems. Furthermore, there are undoubtedly documented examples of natural reductions we have overlooked; this is made more likely by the fact that such reactions are rarely the primary topic of investigation and are thus widely dispersed in the literature.

Several difficulties regarding experimental design and data interpretation have been highlighted. Foremost perhaps, is the question of how one distinguishes between biotic and abiotic processes. Common techniques for the sterilization of reaction media yield ambiguous results. The use of antibiotics is worthy of further consideration [see Atlas (1984) for a discussion of the mode of action of antibiotics].

A second important consideration relates to the redox state of the system. Redox potential (Eh) is clearly inadequate in this regard (see Bohn, 1971; Lindberg and Runnells, 1984; Hostettler, 1984). For example, the studies of the relationship of Eh to rates of nitro reduction reviewed above have no rigorous interpretation. The same problem can be expected in attempts to explain the rates of other natural reduction reactions in terms of measured Eh. Eventually, it will be necessary to characterize all the features that control the redox chemistry of a natural system in far more detail than is usually attempted. Such detail will be very difficult to obtain. However, without such detail, it will not be possible to confidently link the observations of system-level studies into a process-level understanding.

In this regard, it may be fruitful to investigate more thoroughly the role of humic substances in the reduction of anthropogenic substances. Their role in inorganic redox cycles is well known (e.g., Waite, 1985; Thurman, 1985), but to what degree they mediate redox reactions of anthropogenic organics is largely unknown.

Though not independent of the factors discussed above, the effect of solids concentration and composition on reduction rates and mechanisms represents another element in this complex puzzle. Processes involving the transfer of organic molecules between aqueous and solid phases (sorption) are known to have an effect on certain reactions (e.g., Macalady and Wolfe, 1984). Sorption dynamics may also be an important consideration with regard to reduction reactions. The existence of charge transfer complexes between some organic molecules and humic substances may be supportive of this suggestion (Senesi and Testini, 1983, 1984).

Finally, for certain substances, other reaction pathways may complicate the picture. For example, hydrolysis reactions may compete with reduction reactions for methylparathion (Smith et al., 1978), aldicarb (Bank and Tyrrel, 1984), and atrazine (Armstrong and Chesters, 1968). Careful identification of reaction product distribution will be necessary to account for such competing reactions.

SUGGESTIONS FOR FUTURE WORK

Future *system-level* investigations of reductive reactivity in natural anaerobic environments must include a more precise characterization of the system. Rates must be determined; products, intermediates and reducing agents must be identified. Such studies can then provide a basis for *process-level* understanding of the reductive degradations that occur in the anaerobic

natural environment. The phenomena that control such reactions must be defined in terms of basic physical, chemical, geological, and biological principles. Specifically, the parameters that must be determined to adequately characterize the system are: redox state and poising capacity, pH and buffering capacity, iron, manganese, sulfide and trace metal concentrations and speciations, natural organic matter content, mineralogy and morphology of the solid phase, and the size and distribution of the microbial population.

We would like to suggest several hypotheses, which if tested sequentially in future process-level investigations, would lead to a significant clarification of the processes involved in abiotic reductive degradations.

First, reductive degradations require the presence of a solid phase (i.e. sediment). In cases where this hypothesis is false, simplicity can be gained by the use of homogeneous isolates of experimental systems.

Second, reductive degradations are a result of active metabolic, or cometabolic reactions of living cells. Our interest (in a particular reaction) will continue only if this hypothesis is false.

Third, there are reductive degradations caused by extracellular biomolecules (e.g., enzymes, flavins, metal-bioorganic complexes). Where this proves to be true, experiments are needed which are designed to precisely identify the extracellular agent(s) involved.

Fourth, reduced functional groups in humic materials are responsible for the reductive degradation of organic pollutants. Where this proves to be true, characterization of the reactive site(s) in the humic material is desirable.

Fifth, the reductive degradation is affected by strictly inorganic constituents in the sediment—water system. Again, the precise identity of the reducing agent in such circumstances should be pursued.

The conceptual and experimental problems associated with such detailed process-level investigations are, obviously, formidable. The usual approach to characterization of the observed reductive degradation of organic pollutants is deductive in that it involves systematic elimination of reactive agents by purging, chemical blocking, sterilization, etc. Should this approach be taken, we suggest the eliminations be performed in the order of the hypotheses given above. As mentioned in the main body of this review, the side-effects of such procedures generate data that are very difficult to interpret. Perhaps the inductive alternative deserves more attention: Starting with a simple homogeneous model system, various hypotheses can be tested by sequential addition of inorganic reducing agents, humics, extracellular biomolecules, microorganisms, and finally, other solid phases. This approach will circumvent many of the experimental problems associated with the deductive approach.

ACKNOWLEDGMENTS

Work performed in our laboratories mentioned in this review has been supported by cooperative research grants from the Athens, GA and Ada, OK

Environmental Research Laboratories of the U.S. Environmental Protection Agency.

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